Optimal Stoichiometry of PS Gene-editing System

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Abstract

Introduction: The PS Gene-editing System has been shown to increase IDUA expression and improve therapeutic outcomes in murine models of Hurler Syndrome. However, the optimal ratio of bipartite AAV vectors in the system is unknown.

Results: Mice (+/- or -/- for IDUA) treated with a 1:6 ratio of AAV_1:AAV_2 in the PSG System had the highest IDUA activity in plasma and liver, followed by 1:13 and then 1:27 ratio. All treated mice had higher enzyme levels than control mice.

Conclusion: A 1:6 ratio is optimal; however, all ratios resulted in supraphysiological levels of IDUA activity. More research is needed to determine if other ratios (e.g., 1:1), are more effective.

Introduction

Mucopolysaccharidosis type I (MPS I, or Hurler syndrome) is an autosomal recessive disorder marked by a deficiency in the lysosomal enzyme alpha-L-iduronidase (IDUA) and resultant buildup of glycosaminoglycans (GAG) causing severe phenotypes. Treatments include hematopoietic stem cell transplant, which alleviates disease symptoms but carries significant risk, and enzyme replacement therapy, which requires weekly infusions and fails to improve cognitive degeneration.[1,2,3] The PS Gene-editing (PSG) System uses two adeno-associated virus (AAV) vectors to insert a promoterless, functional *IDUA* cDNA into intron 1 of the albumin gene in hepatocytes, allowing for the permanent expression of *IDUA* under the albumin promoter and cross correction to other tissues.[2] Previous work has demonstrated that the PSG System provides therapeutic levels of IDUA, reduced GAG, and induces therapeutic levels neurological outcomes in murine models for MPS I.[2,4] However, the ratio of CRISPR Cas9 and gRNA under a liver specific promoter (AAV_1) to promoterless cDNA donor *IDUA* template (AAV_2) to optimize IDUA expression while reducing Cas9 endonuclease is unknown.

Methods

Vectors: Vectors were prepared according to the investigator's specification by the Children's Hospital of Philadelphia Research Vector Core Laboratory.
Injection: Neonatal mice (+/- or -/- for IDUA) were injected in the facial vein on postnatal day 0-1 with an AAV_1:AAV_2 ratio of 1:6, 1:13, or 1:27.
Assays: Plasma and liver IDUA activity was assayed 4-5 weeks post injection using 4-methylumbelliferyl α-L-iduronide fluorogenic substrate.[5]

Results

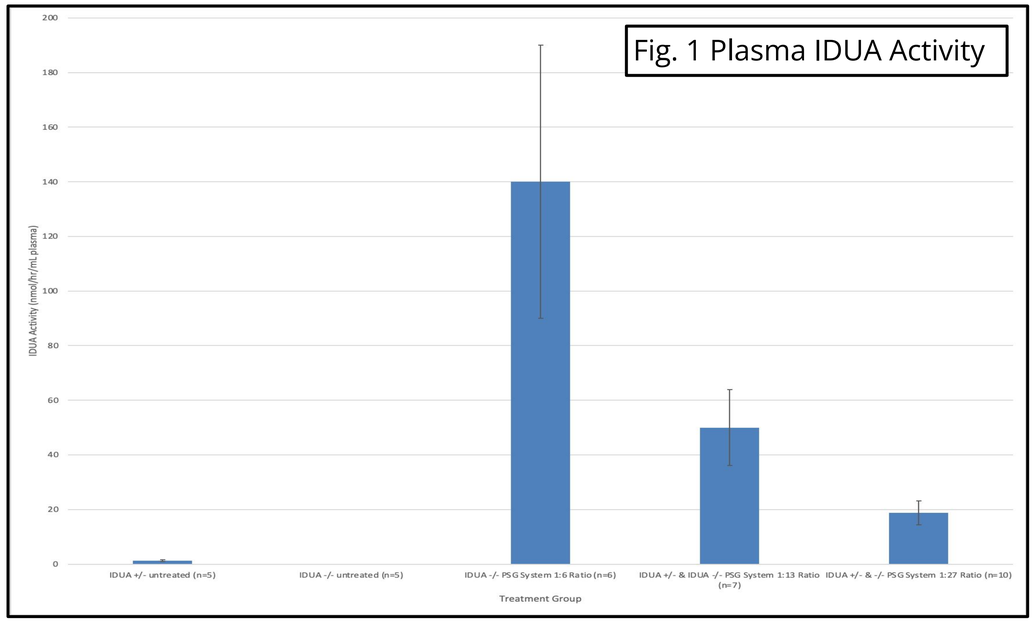

Figure 1: Plasma IDUA activity (nmol/hr/mL plasma) was measured using 4-methylumbelliferyl α-L-iduronide fluorogenic substrate in mice 4-5 weeks following treatment as neonates (P0-P1) with PSG System with AAV_1:AAV_2 of 1:6, 1:13, 1:27, or untreated control. Mice treated with the 1:6 ratio (n=6) had an average IDUA activity of 139.9736 (±50.0084) nmol/hr/mL, compared to 50.0157 (±13.9020, n=7) nmol/hr/mL for the 1:13 group, and 18.80 (±4.3982, n=10) nmol/hr/mL for the 1:27 group. However, treated mice had higher IDUA activity than the heterozygous (IDUA+/-, n=5) and homozygous mutant (IDUA -/-, n=5) controls which were 1.2721 (±0.3199) nmol/hr/mL plasma and 0 nmol/hr/mL plasma respectively.

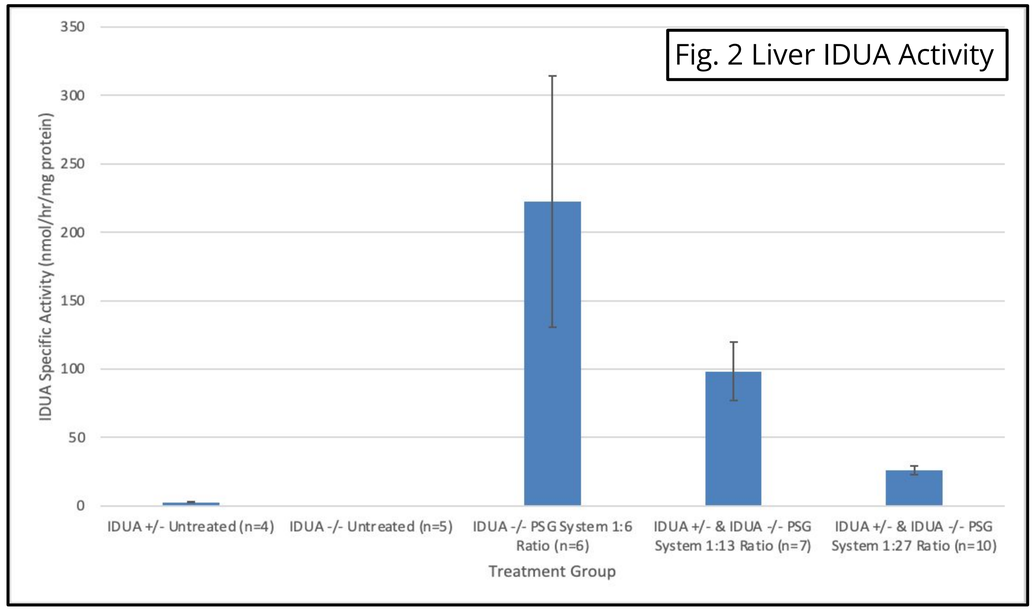

Figure 2: Liver IDUA activity (nmol/hr/mg protein) measured using 4-methylumbelliferyl α-L-iduronide fluorogenic substrate in mice 5 weeks after treatment with PSG System with AAV_1:AAV_2 of 1:6, 1:13, 1:27, or untreated control. For liver, the 1:6 ratio group (n=6) had an average IDUA activity of 222.2684 nmol/hr/mg protein (±91.7412), the 1:13 ratio (n=7) group had activity of 98.4341 nmol/hr/mg protein (±21.6371), the 1:27 (n=10) group had activity of 25.7892 nmol/hr/mg protein (±3.1798), heterozygous control (n=4) 2.7623 nmol/hr/mg protein (±0.3117), and homozygous control (n=5) 0.2376 nmol/hr/mg protein (±0.0642).

Conclusions

The results demonstrated that the PSG System is effective at increasing IDUA levels above physiological levels in mice heterozygous or homozygous for a loss of function mutation in *IDUA*. While all treatment ratios increased IDUA levels, the most effective ratio was the 1:6 AAV_1:AAV_2 ratio. The 1:6 and 1:13 treatments resulting in higher IDUA than 1:27 indicates that a certain amount of CRISPR Cas9 is necessary to optimize integration of *IDUA* in the PSG System. However, a limitation of this study is the variability of IDUA levels within treatment groups. Possible explanations include variable injection success, variability due to the viral vectors which had been repeatedly thawed and frozen, or variability in integration success in different animals.

Previous studies have used 1:6 as the treatment ratio for the PSG System. Data from this experiment indicate that additional ratios, such as 1:3 or 1:1, should be tested to determine if this will further optimize integration or if integration success plateaus. Additionally, GAG accumulation and behavior analysis should be conducted on animals treated at different ratios to determine if plasma/liver IDUA levels correlate with prevention of other disease phenotypes, particularly related to the brain.

References

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